



TITLE:

Overexpression of ETS-1 is associated with malignant biological features of prostate cancer.

AUTHOR(S):

Li, Bo; Shimizu, Yosuke; Kobayashi, Takashi; Terada, Naoki; Yoshimura, Koji; Kamba, Tomomi; Mikami, Yoshiki; Inoue, Takahiro; Nishiyama, Hiroyuki; Ogawa, Osamu

CITATION:

Li, Bo ...[et al]. Overexpression of ETS-1 is associated with malignant biological features of prostate cancer.. Asian journal of andrology 2012, 14(6): 860-863

ISSUE DATE:

2012-11

URL:

<http://hdl.handle.net/2433/175241>

RIGHT:

© 2013 SIMM, CAS and SJTU; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。; This is not the published version. Please cite only the published version.

Overexpression of ETS-1 is associated with malignant biological features of prostate cancer

Bo Li,^{1,2} Yosuke Shimizu,¹ Takashi Kobayashi,^{1,3} Naoki Terada,¹ Koji Yoshimura,¹

Tomomi Kamba,¹ Yoshiki Mikami,⁴ Takahiro Inoue,¹ Hiroyuki Nishiyama,⁵ Osamu

Ogawa,¹

¹Department of Urology, Kyoto University Graduate School of Medicine, Kyoto, Japan,

²Minimally Invasive Urology Center, Shandon Provincial Hospital Affiliated to

Shandon University, Jinan, China, ³Department of Urology, Columbia University

Herbert Irving Cancer Center, New York, the United States, ⁴Department of Diagnostic

Pathology, Kyoto University Hospital, Kyoto, Japan, and ⁵Department of Urology,

Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

Correspondence: Osamu Ogawa M.D., Ph. D., Department of Urology, Kyoto University Graduate School of Medicine, 54 Shogoinkawahara-cho, Sakyo-ku, Kyoto 606-8507 Japan. Email: ogawao@kuhp.kyoto-u.ac.jp

Running title: ETS-1 in advanced prostate cancer

Word count: 2082

Abstract

Objective: To investigate clinical implications of ETS-1 expression in prostate cancer, particularly high-risk cases, including response to androgen deprivation therapy.

Methods: The expression of ETS-1 was analyzed by immunohistochemical staining of paraffin-embedded prostate carcinoma tissue obtained by needle biopsy from 69 mostly advanced prostate cancer patients. ETS-1 expression was compared with clinicopathological characteristics of the 69 patients including 25 who underwent androgen-deprivation therapy as a primary treatment.

Results: Prostate cancers with higher expression of ETS-1 were significantly more likely to be of high stage and high Gleason score ($P<0.05$). There was no significant association between ETS-1 expression and initial prostate-specific antigen (PSA) level. In the 25 patients treated by androgen deprivation therapy, the staining scores for ETS-1 was significantly associated with rapid development of castration-resistant disease within 24 months ($P<0.05$), whereas Gleason sum or PSA level was not.

Conclusions: Increased ETS-1 expression was associated with higher stage, higher Gleason score, and shorter time to castration-resistant progression. These data suggest that immunostaining for ETS-1 can be a molecular marker for predicting poor clinical outcome of prostate cancer patients, particularly with high-risk disease.

Key words: prostate cancer, ETS-1, immunohistochemistry, castration resistant

Introduction

Prostate cancer (PC) is the most commonly diagnosed malignancy among males in the developed world and the second leading cause of cancer-related mortality.¹ Patients with low-risk localized disease can be cured with surgery or radiation, whereas patients with high-risk or advanced disease have a poor prognosis. Although hormonal therapy in the form of medical or surgical castration can induce disease remissions even in these advanced cases, development of castration-resistant prostate cancer (CRPC) is eventually inevitable. In terms of treatment outcome, duration of the disease remission is critical for patient survival since therapeutic options for CRPC are limited and prognosis is poor, with a median survival of less than 2 years.²⁻³ However, there has been limited knowledge of pretreatment prognostic factors for clinical outcome of high-risk or advanced prostate cancer patients.⁴⁻⁵ Thus, there is an urgent need to establish new prognostic markers to aid in the prediction of unfavorable prognostic groups.

E26 transformation-specific-1 (ETS-1), originally characterized as the v-ets retroviral gene, one of the two oncogenes (v-myb and v-ets) in the avian leukemia retrovirus E26, has been reported to play an important role in a variety of physiological and pathological processes, including embryogenesis, wound healing, and tumor

progression, attributed to their ability to regulate the expression of proteins involved in angiogenesis, invasion, and metastasis.⁶⁻⁷ It has been reported that ETS-1 is induced by some growth factor signals including vascular endothelial growth factor (VEGF) and both acidic and basic fibroblast growth factors (FGF)⁸ and activates transcription of various metastasis-, angiogenesis- and invasion-associated genes including matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA), and genes involved in energy metabolisms.^{6,9}

In fact, previous studies showed close associations between ETS-1 expression in the primary tumor and local or metastatic progression of the tumor has been found in human colorectal,¹⁰ breast,¹¹⁻¹² lung,¹³ gastric,¹⁴⁻¹⁵ and ovarian cancers,¹⁶ indicating that ETS-1 expression is a useful marker for predicting metastasis and the outcome in patients with malignancies. In terms of prostate tumor, a recent report showed that ETS-1 could be a tumor marker for the differentiation of latent prostatic carcinoma from clinically significant disease.¹⁷ The authors observed significant increases in ETS-1 expression along with progression through benign, and latent and clinical prostate cancers. They also reported that higher ETS-1 expression was associated with higher histological grade in both latent and clinical PC tissues. A more recent report also showed a significant correlation between ETS-1 expression and

histological grade of prostate cancer.¹⁸ They also showed ETS-1 activation can promote castration-resistant progression of prostate cancer cells. However, clinical significance of ETS-1 expression among relatively advanced prostate cancers is yet to be elucidated. In the present study, we investigated the significance of ETS-1 expression in the prediction of treatment outcome of advanced prostate cancer patients.

Materials and Methods

Samples of prostatic needle biopsy were obtained from 69 patients diagnosed to have PC at Kyoto University Hospital. Clinical charts of the 69 patients were reviewed for their clinicopathological characteristics and most of the cases had a metastatic or high-risk disease as evident in Table 1. This study was approved by the Institutional Review Board of Kyoto University Graduate School of Medicine (G-52) and all patients gave a written informed consent for the use of their tissue samples.

There were 31 patients with metastatic disease, and 25 of them (the mean age at the time of biopsy was 74.6 ± 7.7 years) were available for followup data after androgen deprivation therapy (ADT). The 25 patients were additionally reviewed with regard to the time from the initiation of ADT to the development of CRPC, defined as three consecutive monthly increases in prostate-specific antigen (PSA) against hormonal therapy including antiandrogens.¹⁴ The patients were followed until 31 May 2009, at the median (range) of 69.6 (15 to 114) months. During this followup, 16 (64%) patients developed CRPC within 24 months, while the 9 (36%) were still responsive to ADT at the time of 24 months.

Paraffin-embedded sections of the prostate biopsy specimen from the 69 patients were examined with immunohistochemical (IHC) staining. Material handling and immunostaining were performed according to methods described previously.^{17,19} Antigen retrieval was done with the microwave method. Briefly, citrate buffer (pH 6.0) was boiled for 10 min in a pressure-proof rice cooker (Rakuchin Gozen, Daiya, Tokyo, Japan) by microwave. Glass slides were put into a metal rack and boiled for another 20 min in the rice cooker. Endogenous peroxidase activity was blocked by 0.3% H₂O₂ in methyl alcohol for 30 min. ETS-1 (C-20) affinity purified rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA.) was used at a dilution of 1:500. We confirmed, using western blotting, that the antibody recognizes ETS-1 but not ERG, ETV1 or ETV4 (data not shown). After overnight incubation with primary antibody at 4°C, samples were then incubated with biotinylated anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA, USA) diluted to 1:300 for 40 min. Avidin–biotin–peroxidase complex (ABC; ABC-Elite, Vector Laboratories) at a dilution of 1:100 was applied for 50 min. The coloring reaction was carried out with 0.3 mg/mL of diaminobenzidine and 0.003% H₂O₂ and nuclei were counterstained with hematoxylin. For negative control, the primary antibody was omitted.

The histopathological examination for ETS-1 expression and Gleason score was

performed by two of the authors (Y.S. and Y.M.) who were blinded to the clinicopathological data. ETS-1 expression levels were graded as 0, 1 and 2 based on staining intensity to represent from no staining to strong staining.

The Pearson chi-square test or Fisher's exact probability test was used to analyze the association between the expression of ETS-1 and Gleason score, tumor stage, patients' age and PSA level as appropriate. All statistical analyses were performed using SPSS 16.0 software and $P < 0.05$ was considered statistically significant.

Results

IHC for ETS-1 demonstrated signals both in the nucleus and cytoplasm of prostatic tumor cells (Figure 1) but not in the adjacent benign tissues. There was a highly significant difference in the staining scores for ETS-1 among tumors at different clinical stage ($P = 0.04$; Table 2). Interestingly, 8 (89%) of 9 patients with score 2 were locally advanced or metastatic tumors. There was a statistically significant association among the staining scores for ETS-1 and the tumor's Gleason sum ($P = 0.03$). There was no significant association of the staining scores for ETS-1 with PSA levels ($P = 0.11$) and age ($P = 0.91$).

For the 25 patients with metastatic PC treated by ADT, there was a significant difference in the staining scores for ETS-1 between patients with and without rapid progression to CRPC within 24 months ($P = 0.016$; Table 3), but not in the Gleason sum ($P = 0.225$) or initial PSA level ($P = 0.364$).

Discussion

In the present study IHC staining showed that ETS-1 expression was located to both nucleus and cytoplasm, which is consistent with previous reports.¹⁰⁻¹² The reliability of immunohistochemistic techniques for the purpose to examine ETS-1 expression was supported by the well correlated results of *in situ* hybridization.¹⁰⁻¹¹ These collectively indicate that IHC technique used in the present study is a reliable and reproducible method for evaluating the expression of functional ETS-1 protein.

Using this technique, we have demonstrated that ETS-1 expression is associated with clinical stage and Gleason grade of PC. About 45% (13/29) of the PC with no ETS-1 expression (staining score 0) were clinically localized diseases, whereas 83% (33/40) of those with higher expression (staining score 1-2) were advanced (Table 2). Additionally, all cases except one (97%) of those with higher ETS-1 expression contained high-grade cancer component (Gleason score 7 or more). Our findings strongly suggest that ETS-1 expression is significantly associated with the aggressive biological features of PC, and therefore, associated with the adverse clinicopathological characteristics of PC.

Additionally, we have shown that ETS-1 expression is significantly associated with the rapid castration-resistant progression of PC after androgen deprivation therapy.

This is consistent with a previous report by Smith et al.¹⁸ showing that ETS-1 activation led prostate cancer cells to castration-resistant status. Our results suggest that ETS-1 expression can be a useful predictor for treatment outcome of the patients with advanced prostate cancer. A major clinical problem with hormonal therapy of advanced PC is the pretreatment prediction of time to castration-resistant progression.

Recently, Sim et al.²⁰ reported that failure to attain a nadir PSA of < 1 ng/mL after treatment was the most important predictor of time to CRPC and a nadir PSA of > 2 ng/mL was a risk factor for poor overall survival. However, those findings will be obtained only after treatment has been initiated, and as the authors reported, prediction of treatment outcome at pretreatment settings was difficult since the biopsy Gleason score or initial PSA level was not a predictor of rapid progression to CRPC. Indeed, the biopsy Gleason score and initial PSA level failed to predict time to progression to CRPC in the present study, too. In this regard, a significant association between increased ETS-1 expression and a shorter time to be CRPC observed in this study would enable us to separate those patients who are likely to fail on hormone therapy from those who are more likely to achieve long term stable disease at pretreatment settings. This is an important prospect, which should be validated with a larger study in the future.

Aberrant activation of Ets family transcription factors by gene fusion with certain androgen-dependent genes is a frequently observed alteration inducing invasion of prostate cancer cells,²¹⁻²² which is now considered as an initiation²³ and progression²⁴ events in the natural history of PC. Considering that Ets family transcription factors share a number of target genes, ETS-1 is likely to play an important role in prostate cancer. Indeed, a number of genes that can facilitate cancer progression are known as transcriptional target of ETS-1.^{6,9} Additionally, ETS-1 has been reported to activate transcription of Sp1 resulting in an increase in lipogenesis and cell proliferation through induction of fatty acid synthase,²⁵ which is thought to be associated with castration-resistant progression of PC.²⁶

We recognize that this study has a limitation of the relatively few patients, although other strengths were comprehensive data collection, analysis by a central pathologist and a reasonable duration of follow-up, which helped to compensate for the small samples. Prospective validation studies on ETS-1 expression together with other molecular markers are warranted in the future. Using biopsy tissue can be problematic as tumor often shows heterogeneity. However, clinical decision-making should be often made based on biopsy specimen. As it has been reported previously that the antibody used in this study can cross-react with Annexin V, an unrelated protein.²⁷ Although we

cannot exclude strictly the possibility that the antibody recognize unrelated protein, ETS-1 expression evaluated with the antibody still has diagnostic significance in predicting time to castration-resistant progression.

In conclusion, immunohistochemical analysis of PC specimens clearly demonstrated that the expression of ETS-1 was associated with pathological grade and clinical stage of the disease. Importantly, we found that ETS-1 expression was significantly associated with time to CRPC in patients with advanced PC. Although further systematic studies are necessary, these findings support the possibility that the higher expression of ETS-1 can play an important role in the poor differentiation of PC cells. Thus, ETS-1 may be a useful marker for the risk of PC progression.

Acknowledgements

This study was partly supported by a Grant-in-Aid from the Ministry of Education,
Culture, Sports, Science, and Technology of Japan, Takeda Science Foundation (to
B.L.), and The Uehara Memorial Foundation (to. T. Ko. and O.O.).

Reference

- 1 Siegel, R., Ward, E., Brawley, O. & Jemal, A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* **61**, 212-236, doi:caac.20121 [pii] 10.3322/caac.20121 (2011).
- 2 Tannock, I. F. et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* **351**, 1502-1512, doi:351/15/1502 [pii] 10.1056/NEJMoa040720 (2004).
- 3 Petrylak, D. P. et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* **351**, 1513-1520, doi:351/15/1513 [pii] 10.1056/NEJMoa041318 (2004).
- 4 Lu-Yao, G. L. et al. Survival following primary androgen deprivation therapy among men with localized prostate cancer. *JAMA* **300**, 173-181, doi:300/2/173 [pii] 10.1001/jama.300.2.173 (2008).
- 5 DiBlasio, C. J. et al. Survival outcomes in men receiving androgen-deprivation therapy as primary or salvage treatment for localized or advanced prostate cancer: 20-year single-centre experience. *BJU Int* **104**, 1208-1214, doi:BJU8593 [pii] 10.1111/j.1464-410X.2009.08593.x (2009).
- 6 Dittmer, J. The biology of the Ets1 proto-oncogene. *Mol Cancer* **2**, 29, doi:10.1186/1476-4598-2-29 1476-4598-2-29 [pii] (2003).
- 7 Oikawa, T. ETS transcription factors: possible targets for cancer therapy. *Cancer Sci* **95**, 626-633 (2004).
- 8 Iwasaka, C., Tanaka, K., Abe, M. & Sato, Y. Ets-1 regulates angiogenesis by inducing the expression of urokinase-type plasminogen activator and matrix metalloproteinase-1 and the migration of vascular endothelial cells. *J Cell Physiol*

- 169**, 522-531,
doi:10.1002/(SICI)1097-4652(199612)169:3<522::AID-JCP12>3.0.CO;2-7 [pii]
10.1002/(SICI)1097-4652(199612)169:3<522::AID-JCP12>3.0.CO;2-7 (1996).
- 9 Verschoor, M. L., Wilson, L. A., Verschoor, C. P. & Singh, G. Ets-1 regulates energy metabolism in cancer cells. *PLoS One* **5**, e13565,
doi:10.1371/journal.pone.0013565 (2010).
 - 10 Nakayama, T., Ito, M., Ohtsuru, A., Naito, S. & Sekine, I. Expression of the ets-1 proto-oncogene in human colorectal carcinoma. *Mod Pathol* **14**, 415-422,
doi:10.1038/modpathol.3880328 (2001).
 - 11 Behrens, P., Rothe, M., Wellmann, A., Krischler, J. & Wernert, N. The Ets-1 transcription factor is up-regulated together with MMP 1 and MMP 9 in the stroma of pre-invasive breast cancer. *J Pathol* **194**, 43-50, doi:10.1002/path.844 [pii]
10.1002/path.844 (2001).
 - 12 Buggy, Y. et al. Overexpression of the Ets-1 transcription factor in human breast cancer. *Br J Cancer* **91**, 1308-1315, doi:10.1038/sj.bjc.6602128 6602128 [pii]
(2004).
 - 13 Sasaki, H. et al. Clinical significance of matrix metalloproteinase-7 and Ets-1 gene expression in patients with lung cancer. *J Surg Res* **101**, 242-247,
doi:10.1006/jsre.2001.6279 S0022-4804(01)96279-1 [pii] (2001).
 - 14 Tsutsumi, S. et al. Ets-1 expression in gastric cancer. *Hepatogastroenterology* **52**, 654-656 (2005).
 - 15 Yu, Y. et al. Ets1 as a marker of malignant potential in gastric carcinoma. *World J Gastroenterol* **9**, 2154-2159 (2003).
 - 16 Takai, N., Miyazaki, T., Nishida, M., Nasu, K. & Miyakawa, I. c-Ets1 is a

- promising marker in epithelial ovarian cancer. *Int J Mol Med* **9**, 287-292 (2002).
- 17 Alipov, G. et al. Overexpression of Ets-1 proto-oncogene in latent and clinical prostatic carcinomas. *Histopathology* **46**, 202-208, doi:HIS2059 [pii] 10.1111/j.1365-2559.2005.02059.x (2005).
- 18 Smith, A. M. et al. ETS1 transcriptional activity is increased in advanced prostate cancer and promotes the castrate-resistant phenotype. *Carcinogenesis* **33**, 572-580, doi:bgs007 [pii] 10.1093/carcin/bgs007 (2012).
- 19 Inoue, T. et al. Androgen receptor, Ki67, and p53 expression in radical prostatectomy specimens predict treatment failure in Japanese population. *Urology* **66**, 332-337, doi:S0090-4295(05)00305-5 [pii] 10.1016/j.urology.2005.02.028 (2005).
- 20 Sim, H. G., Lau, W. K. & Cheng, C. W. Predictors of androgen independence in metastatic prostate cancer. *BJU Int* **93**, 1221-1224, doi:10.1111/j.1464-410X.2004.04863.x BJU4863 [pii] (2004).
- 21 Tomlins, S. A. et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310**, 644-648, doi:310/5748/644 [pii] 10.1126/science.1117679 (2005).
- 22 Sreekumar, A. et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* **457**, 910-914, doi:nature07762 [pii] 10.1038/nature07762 (2009).
- 23 Tomlins, S. A. et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* **448**, 595-599, doi:nature06024 [pii] 10.1038/nature06024 (2007).
- 24 Carver, B. S. et al. ETS rearrangements and prostate cancer initiation. *Nature* **457**,

- E1; discussion E2-3, doi:nature07738 [pii] 10.1038/nature07738 (2009).
- 25 Lu, S. & Archer, M. C. Sp1 coordinately regulates de novo lipogenesis and proliferation in cancer cells. *Int J Cancer* **126**, 416-425, doi:10.1002/ijc.24761 (2010).
- 26 Segawa, T. et al. Androgen-induced expression of endoplasmic reticulum (ER) stress response genes in prostate cancer cells. *Oncogene* **21**, 8749-8758, doi:10.1038/sj.onc.1205992 (2002).
- 27 Dittmer, J. Does a truncated form of the transcription factor Ets1 exist in breast cancer cells? *Br J Cancer* **94**, 176-177, doi:6602903 [pii] 10.1038/sj.bjc.6602903 (2006).

Figure legend

Figure 1. Immunohistochemistry of ETS-1 in prostatic carcinomas. It should be noted that heterogeneous expression of ETS-1 is observed both in the cytoplasm and the nucleus of the tumor cells. (a) No staining (staining score 0). (b) Weak staining (staining score 1). (c) Strong staining (staining score 2). (d) Negative control. Scale bar = 50.0 μm .

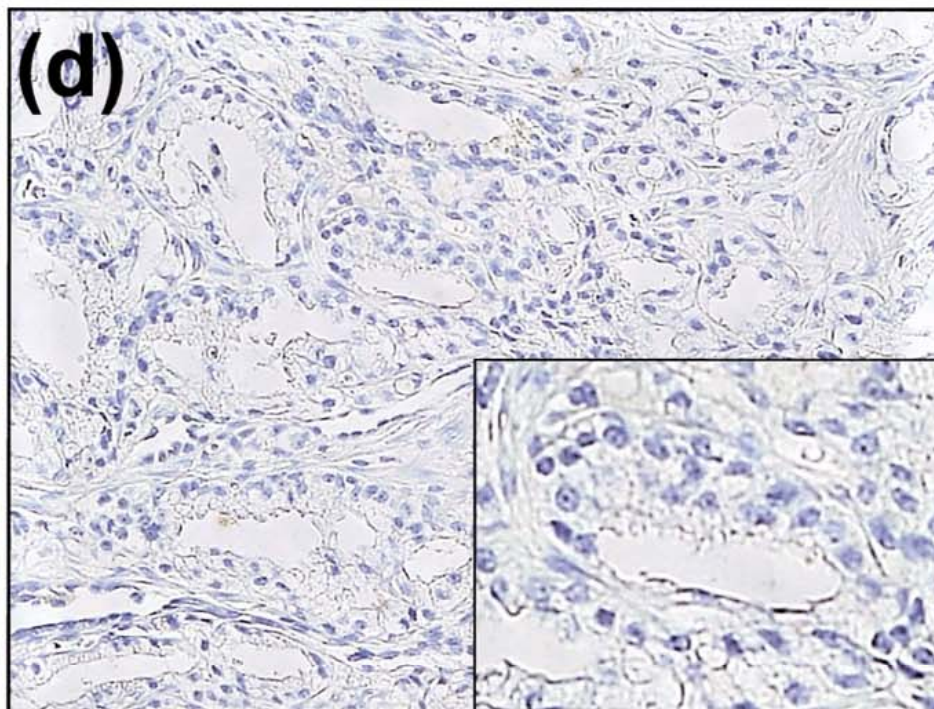
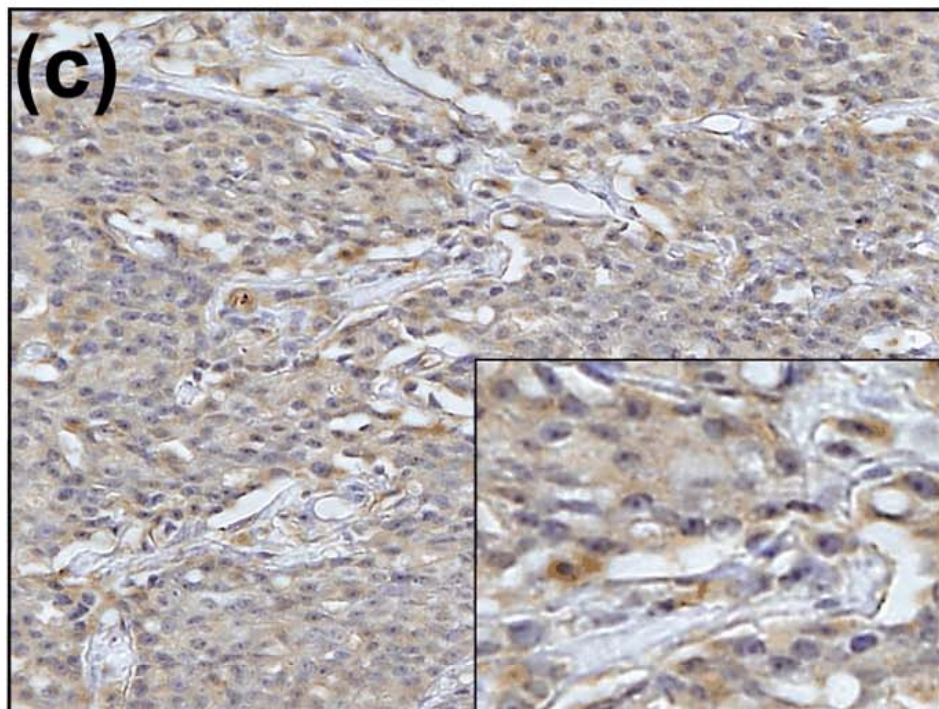
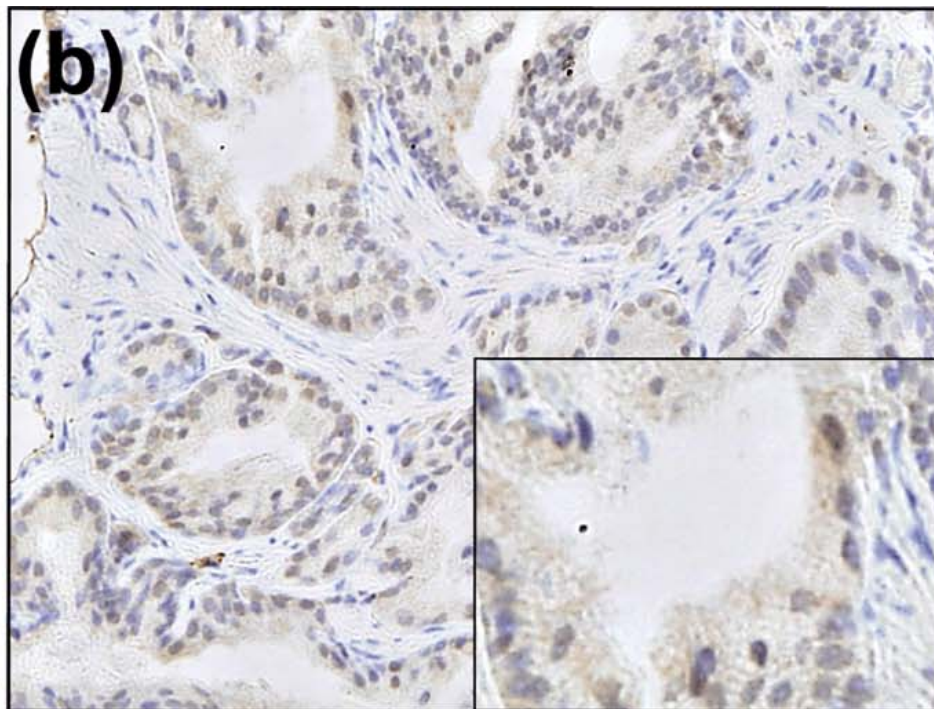
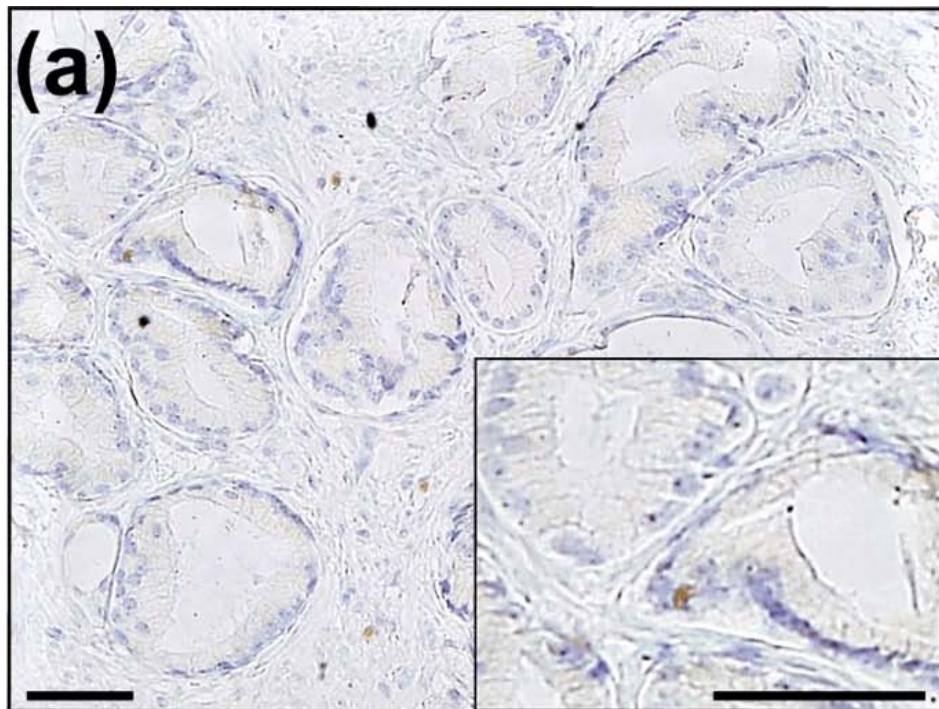


Table 1 Clinicopathological characteristics of the 69 prostate cancer patients

Variable	Median (range) or N (%)
Age (years)	72.4 (54-92)
≤70	27 (39.1%)
>70	42 (60.9%)
Clinical stage	
Localized	20 (29.0%)
Locally advanced	18 (26.1%)
Metastatic	31 (44.9%)
Gleason score	
6 or less	8 (11.6%)
7	32 (46.4%)
8-10	29 (42.0%)
Initial PSA (ng/ml)	426.9 (4.2-13000)
<10	18 (26.1%)
10-20	14 (20.3%)
>20	37 (53.6%)

Table 2. Expression of ETS-1 in relation to clinicopathological characteristics.

Variable	Number of cases	ETS-1 expression (score)			<i>P</i> value*
		0 (%)	1 (%)	2 (%)	
		(n = 29)	(n = 31)	(n = 9)	
Clinical stage					
Localized	20	13 (65)	6 (30)	1 (5)	<i>P</i> = 0.04
Locally advanced	18	8 (44)	6 (33)	4 (22)	
Metastatic	31	8 (26)	19 (61)	4 (13)	
Gleason score					
6 or less	8	7 (88)	1 (12)	0 (0)	<i>P</i> = 0.03
7	32	17 (53)	11 (34)	4 (13)	
8-10	29	5 (17)	19 (66)	5 (17)	
Age (years)					
≤70	27	12 (44)	12 (44)	3 (11)	<i>P</i> = 0.91
>70	42	17 (40)	19 (45)	6 (14)	
Initial PSA (ng/ml)					
<10	18	8 (44)	8 (44)	2 (11)	<i>P</i> = 0.11
10-20	14	9 (64)	2 (14)	3 (21)	
>20	37	12 (32)	21 (57)	4 (11)	

* Chi-square test

Table 3. Association between ETS-1 expression and the time to be CRPC in 25 metastatic prostate cancer patients treated by ADT.

Variable	Number of cases	Time to be CRPC		<i>P</i> value*
		>24 months	<24 months	
		(n = 9)	(n = 16)	
ETS-1 expression (score)				
0	10	7	3	<i>P</i> = 0.016
1	11	2	9	
2	4	0	4	
Gleason score				
6 or less	2	2	0	<i>P</i> = 0.225
7	8	2	6	
8-10	15	5	10	
Initial PSA (ng/ml)				
<500	19	8	11	<i>P</i> = 0.364**
>500	6	1	5	

* Chi-square test, **Fisher's exact test